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Effect of renal vein constriction on the localization of immune complexes in the kidney

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Effect of renal vein constriction on the localization of immune complexes in the kidney. To determine the effect of renal venous constriction on the localization of immune complexes in the kidney, we performed unilateral renal venous constriction in 19 male albino rabbits 7 days after they were given an i.v. bolus (250 mg/kg) of bovine serum albumin (BSA) to induce the development of serum sickness nephritis. The rabbits were sacrificed on day 12 after BSA administration. Renal histology was evaluated by (*1*) light microscopy, with semiquantitative grading and enumeration of glomerular nuclei and (*2*) fluorescent microscopy. Six rabbits were nonresponders and did not exhibit nephritis. The remaining 13 rabbits had differential degrees of severity of nephritis between the control and experimental kidneys as assessed by light microscopy (in 7 rabbits) and by fluorescent microscopy (in 12 and in 9 rabbits when examined for deposits of IgG and C3, respectively). In all instances, the experimental kidney (with the constricted vein) showed less severe alterations. The degree of protection appeared to correlate with the degree of renal venous constriction, as manifested by tubulointerstitial changes [$r_s = 0.77$, $P < 0.01$). We concluded that renal venous constriction exercises a protective effect on the impaction of immune complexes in the glomeruli and development of acute serum sickness nephritis.

Effet de la constriction de la veine rénale sur la localisation des complexes immuns dans le rein. Afin d'étudier l'effet de la constriction de la veine rénale sur la localisation des complexes immuns dans le rein, une constriction veineuse unilatérale a été réalisée chez 19 lapins mâles, albinos, sept jours après qu'ils aient reçu une injection i.v. unique (250 mg/kg) de sérum albumine bovine (BSA) afin de déterminer le développement d'une néphrite de la maladie sérique. Les animaux ont été sacrifiés le douzième jour après l'administration de BSA. L'histologie rénale a été étudiée par (*1*) la microscopie photonique, avec évaluation semiquantitative et un dénombrement des noyaux glomérulaires, et (*2*) la fluorescence. Six parmi ces lapins n'ont pas fait de néphrite. Parmi les 13 lapins restants des différences de degré dans la sévérité de la néphrite ont été constatées entre les reins contrôles et expérimentaux comme en témoignent la microscopie photonique (7 lapins) et la fluorescence (12 et 9 lapins chez lesquels ont été étudiés, respectivement, les dépôts d'IgG et de C3). Dans tous les cas, le rein expérimental (celui dont la veine avait subi une constriction) montre des modifications moindres. Le degré de protection paraît être corrélé avec le degré de constriction de la veine rénale, lui-même traduit par des modifications tubulointerstitielles ($r_s = 0.77$, $P < 0.01$). Il est conclu que la constriction veineuse rénale exerce un effet protecteur vis à vis de la fixation de complexes immuns sur le glomérule et du développement de la néphrite au cours de la maladie sérique aiguë.

The association of renal vein thrombosis with the nephrotic syndrome due to immune-complex-mediated nephritis is well recognized [1–4]. The underlying type of renal disease has included membranous nephropathy, membranoproliferative glomerulonephritis, and diffuse proliferative glomerulonephritis [1–4]. Although it is still uncertain which is the antecedent event, there is persuasive evidence derived from experimental and clinical studies that in some instances the vascular event probably is superimposed on the nephritis [2, 4, 5]. These considerations prompted us to examine if the development of venous occlusion would influence the deposition of immune complexes in the kidney. We have used a model of unilateral renal venous constriction to allow comparisons to be made within the same animal; constriction rather than total occlusion was used, because total occlusion would give rise to severe hemorrhagic infarction in the kidney, making interpretation of renal histology difficult. (It also is apparent that in the evolution of thrombosis, narrowing of the lumen of the vessel must occur before total occlusion supervenes, so that this model simulates many of the clinical events of renal vein thrombosis.) Using a model of acute serum sickness in the rabbit, the present study shows that renal venous constriction has a protective effect on the localization of immune complexes in the kidney.

Methods

Experimental design. We used 19 male albino rabbits that weighed 2.0 to 2.5 kg. They were fed Purina rabbit pellets and water ad lib. Each animal was immunized with an i.v. bolus of bovine serum

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albumin (BSA; 250 mg/kg) dissolved in isotonic saline buffered to a pH of 7.4. On day 7 after BSA administration, renal venous constriction was produced as follows: The animals were anesthetized with an i.m. injection of 25 mg/kg of xylazine and 40 mg/kg of ketamine hydrochloride, followed by inhalation anesthesia with halothane. The left renal vein was exposed by a midline incision, and constriction was obtained by securing two ligatures a few millimeters apart around the vessel, to which a PE-50 tube had been applied. Then, the PE-50 tube was removed, leaving a portion of the vein constricted by the ligatures in place. The abdomen was closed, and the animals were returned to the cages.

The animals were sacrificed on day 12 after BSA administration. This time of sacrifice was chosen because previous studies by others have indicated that a greater proportion of animals would develop the more severe form of nephritis in this time period [6]. A pilot study by us of 7 animals sacrificed on day 9 after BSA administration (after unilateral venous constriction) showed the rabbits developing only minor glomerular abnormalities, disallowing any meaningful comparisons. During sacrifice, the ligatures were inspected to determine if they were in place. Renal venous constriction was assessed as having been obtained, for in all instances the affected kidney was enlarged, its weight being increased to 130% to 190% of the control kidney. Portions of both kidneys were obtained by excision biopsy and processed for light and fluorescent microscopy by previously described techniques [7]. For fluorescent microscopy, we used fluorescein-conjugated monospecific goat antirabbit IgG (Calbiochem-Behring Corp., La Jolla, California) and goat antirabbit C3 (Cappel Laboratories, Cochranville, Pennsylvania).

Methods of histologic evaluation. Sections were assessed by two independent observers, who had no knowledge of which kidney had been removed from the constricted vein; paired kidneys (control and experimental) always were examined on the same occasion.

Light microscopy sections were cut 2 to 3 μ in thickness and stained with hematoxylin and eosin, Masson's trichrome, silver methenamine, and Lendrum stains. By light microscopy, glomerular histology was analyzed by two methods: *(1)* We graded the degree of mesangial and endothelial (and monocyte) hypercellularity on a semiquantitative scale ranging from 0 to 3+ (0 = normal, 1+ = mild, 2+ = moderate, 3+ = severe). *(2)* After a lapse of 2 months, the sections were reexamined with glomerular nuclear counts done on 20 glomeruli that were cut through the vascular pole. The mean glomerular nuclear counts (± SEM) in relation to the semiquantitative scale was as follows: 0 = 55.3 ± 0.68; 1+ = 62.7 ± 0.8; 2+ = 69.3 ± 1.7; 3+ = 81.9 ± 1.5. The differences between the means of succeeding categories (for example, 1+ vs. 2+) as assessed by Student's t tests were significantly different ($P < 0.01$).

To evaluate alterations secondary to renal vein constriction, we graded tubulointerstitial changes as follows: 0 = no change; ± = minor focal interstitial inflammation without tubular alteration; + = perivenular inflammation with nonspecific tubular epithelial degeneration and cast formation; 2+ = perivenular and peritubular edema and inflammation with tubular dilatation and epithelial flattening; 3+ = acute tubular necrosis; 4+ = acute tubular necrosis with focal hemorrhagic infarction.

Fluorescent microscopy sections were assessed by grading each glomerulus on a scale of 0 to 4, according to the number of quadrantic segments with significant deposition of the immune reactant. For example, a glomerulus with two segments involved was scored as 2+. A mean value was derived from the aggregate scores of 20 glomeruli.

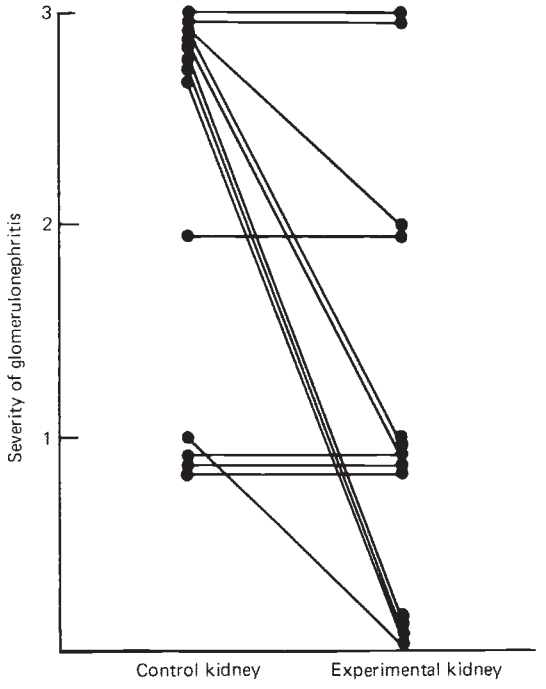

Fig. 1. *Severity of glomerular changes in control and experimental kidneys.* Note a reduction in the severity of glomerulonephritis of 7 kidneys with a constricted vein. Six rabbits (not shown) exhibited no evidence of nephritis.

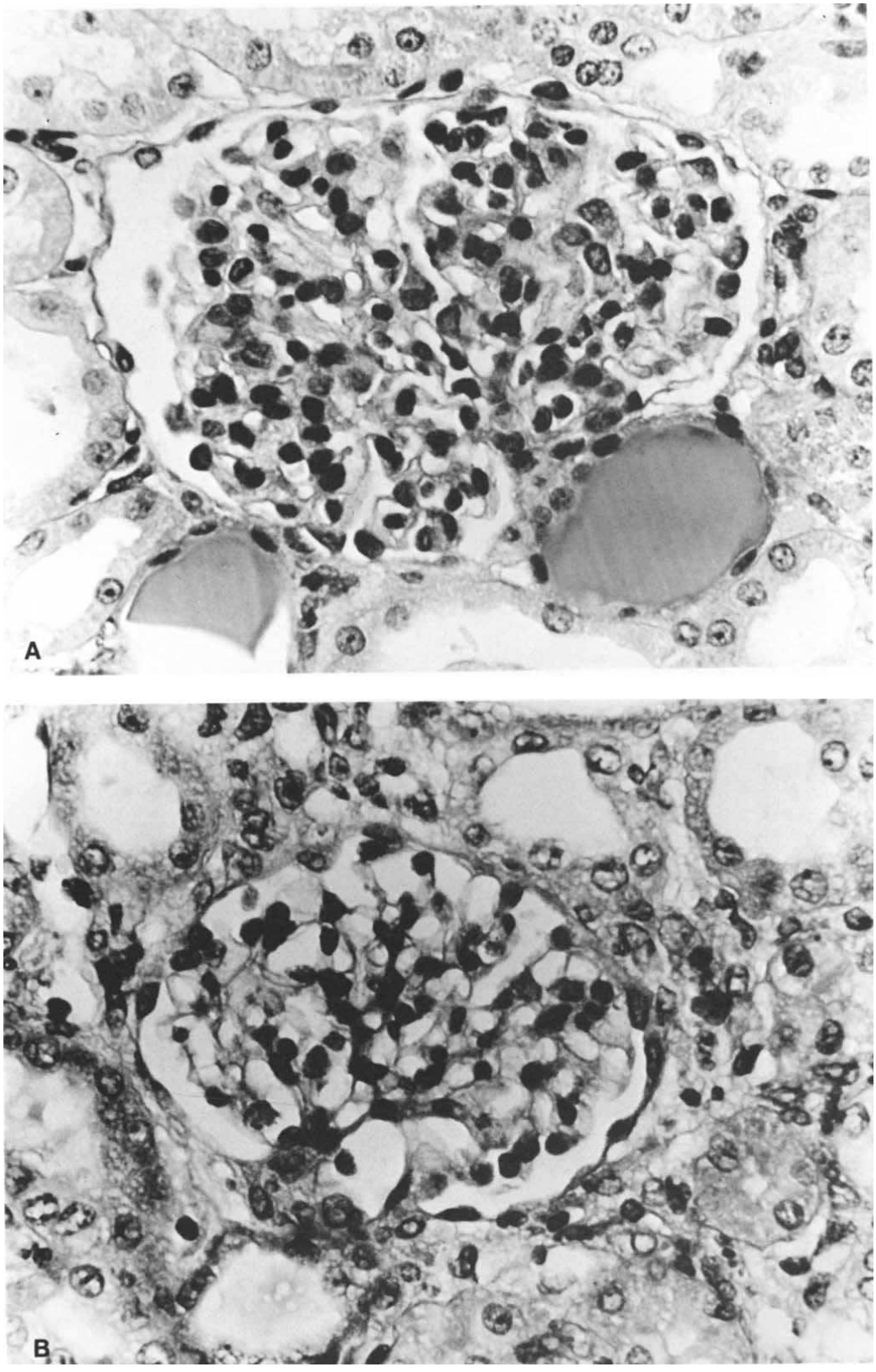

Fig. 2. *Comparison of severity of glomerulonephritis between control* (panels a and c) *and experimental* (panels b and d) kidneys in a rabbit assessed by light and fluorescent microscopy. (×350).

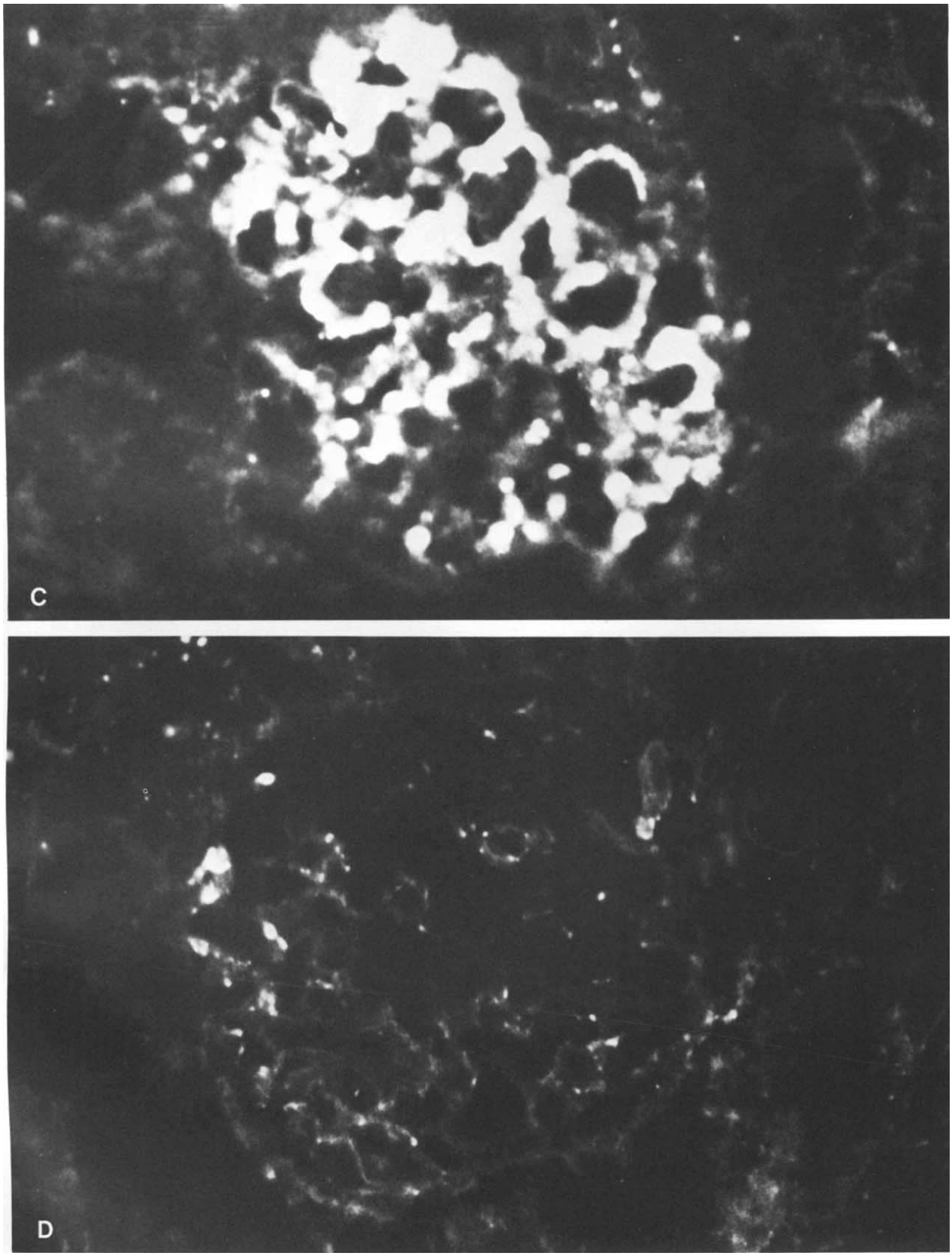

Fig. 2. *Continued.*

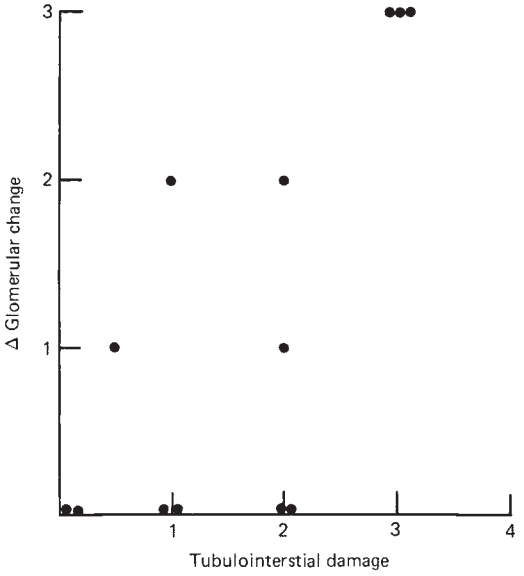

Fig. 3. *Relationship between differential glomerular change (Δ glomerular change) between control and experimental kidney and the degree of tubulointerstitial damage.* The two parameters are significantly correlated by the Spearman-Rank test ($r_s = 0.77$, $P < 0.01$). Only the kidneys exhibiting evidence of nephritis (13 rabbits) are plotted.

Statistical evaluation. Correlation coefficients between various parameters were derived by the Spearman-Rank test. Differences between the mean nuclear counts of control and experimental kidneys were assessed by Student's paired *t* test.

Results

Light microscopy. The results are shown in Fig. 1. Of the 19 rabbits, 6 exhibited no glomerular abnormalities. Of the 13 rabbits developing nephritis, 7 had discernible histologic differences between the control and experimental kidneys, the lesions being more severe in the control kidneys. In 5 instances, the differences were marked, being more than two grades of severity. An example of this pronounced differential change is given in Fig. 2. The remaining 6 animals exhibited varying degrees of glomerular damage without any notable differences in degree of severity of nephritis being observed between the control and experimental kidneys. The average glomerular nuclear counts (± SEM) of the control and experimental kidneys were 75.2 ± 4.4 and 64.7 ± 3.0, respectively. By the paired *t* test, the differences between the means were statistically significant (t = 3.14, $P < 0.01$).

Tubulointerstitial abnormalities correlated with the degree of differential glomerular damage (Δ change) between control and experimental kidneys (Fig. 3); the findings show a concordance between the two parameters ($r_s = 0.77$, $P < 0.01$), indicating a relationship between efficacy of renal venous constriction and degree of protection conferred on the experimental kidney.

Immunofluorescent microscopy. The results are shown in Fig. 4. The six rabbits without histologic evidence of nephritis (by light microscopic examination) also did not reveal glomerular deposits of

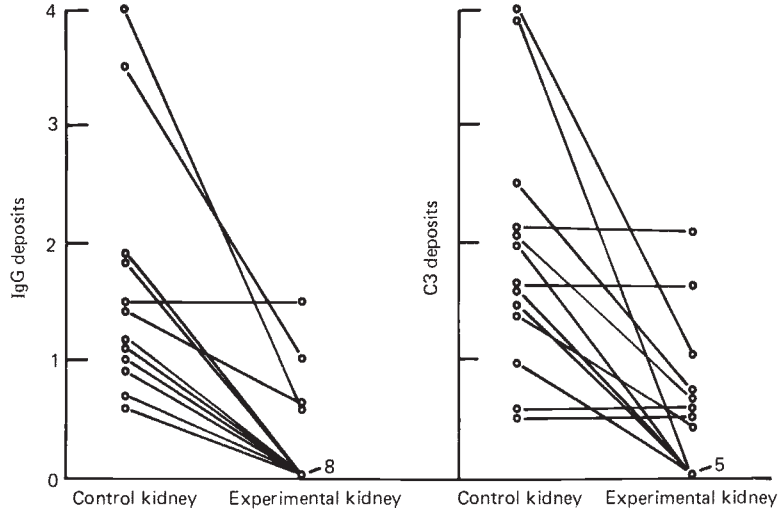

Fig. 4. *Comparison of extent of glomerular deposition of rabbit IgG and rabbit C3 in control and experimental kidneys.* The scale reflects the extent (and not intensity) of glomerular deposition. Note a reduction in the amount of IgG and C3 deposited in 12 and 9 kidneys, respectively. Six rabbits did not reveal evidence of any deposits in either kidney and have not been included.

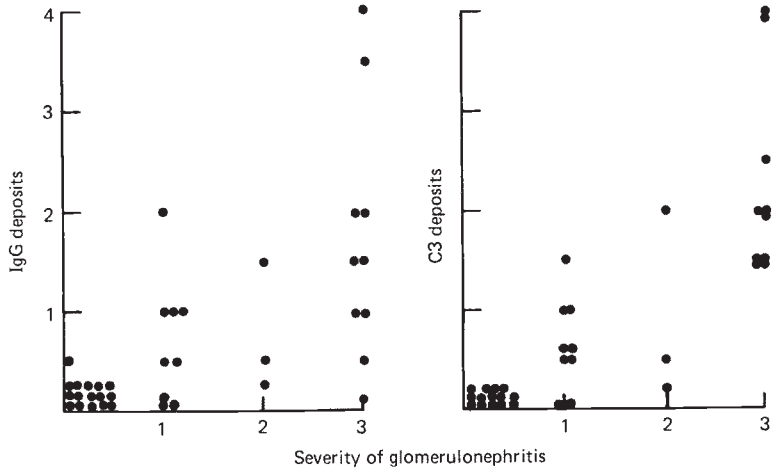

Fig. 5. *Correlation between severity of glomerulonephritis as assessed by light microscopy and amount of IgG and C3 deposited.* Significant correlations were obtained by the Spearman-Rank test for IgG ($r_s = 0.72$, $P < 0.01$) and for C3 ($r_s = 0.80$, $P < 0.01$). The plot includes all 38 kidneys examined.

IgG and C3 by fluorescent microscopy. Of the remaining 13 rabbits, patterns of IgG and C3 deposition were significantly different between control and experimental kidneys in 12 and in 9 rabbits, respectively, with striking differences being observed in 2 rabbits. In the case of IgG deposition, glomeruli from 8 of the experimental kidneys were completely negative, whereas those from the corresponding control kidneys contained varying amounts of this immune reactant, ranging from a score of 0.5 to 4. With respect to C3 deposition, 5 of the kidneys with a constricted vein were completely devoid of this protein in their glomeruli; its presence could be distinctly visualized in the corresponding control kidneys. A positive correlation between severity of histologic nephritis (as assessed by light microscopic examination) and amounts of IgG ($r_s = 0.72$, $P < 0.01$) and C3 ($r_s = 0.80$, $P < 0.01$) deposited was demonstrable (Fig. 5).

Discussion

The present study defines a protective effect of renal venous constriction on the impaction of immune complexes in the kidney. The use of a model of unilateral renal venous constriction allowed us to make comparisons in the same animal, thereby excluding the influence of other determinants such as levels and properties of complexes, which would have to be taken into account if a control and an experimental group of animals had been studied. The validity of the methods used and the nature of the results obtained merit comment. We used two methods to evaluate the glomerular histology by light microscopy, with significant concordance in the results obtained. Objective measurement by glomerular nuclear counts substantiated the method of semiquantitative assessment, with statistically significant differences between the means of the two groups (control and experimental) being observed. Fluorescent microscopy is a technique which is more difficult to use semiquantitatively. Comparisons, however, were facilitated by the complete absence of any deposits of IgG and C3 in 8 and in 5 of the experimental kidneys, respectively, so that quantitation was not a problem in these evaluations. Additionally, 2 rabbits exhibited marked differences in the amounts deposited in their kidneys so that definite differential changes were noted by this technique in 10 rabbits for IgG and in 7 rabbits for C3. Finally, there was a significant concordance between the results obtained by light and fluorescent microscopy.

It is also apparent that differences were not detected in the kidneys of some rabbits. This may reflect the adequacy of venous constriction in these animals; credence for this explanation was given by studies showing a positive correlation between degree of tubulointerstitial alteration and magnitude of differential glomerular change between the control and the affected kidney. (For this reason, sham-operated controls with only gentle manipulation of the renal vein have not been done, because in a number of animals, differential histologic changes and immune complex localization (Figs. 1 and 4, respectively) were not noted despite unilateral renal vein constriction in these animals; it may be inferred that

the gentler procedure would not result in significant differential alterations.)

The mechanism responsible for these differences in the localization of immune complexes in the kidney is uncertain. There is scanty data regarding the influence of various physiologic factors modulating the deposition of immune complexes. Previous studies have documented that complexes tend to be localized in regions of high turbulance, such as sites above and below a constricted vessel [8]. Additionally, Germuth, Kelemen, and Pollack have found that renal arterial constriction and ureteric ligation retard the entrapment of complexes in the glomerulus [9]. Each of these maneuvers provokes a variety of physiologic changes in the kidney, any one of which may be responsible for the effect. In the case of renal vein constriction, it is reasonable to suggest that this effect may in part be due to the redistribution of renal blood flow from the cortical to the medullary regions; this phenomenon has been observed to occur with manipulation of the renal vein [10]. Transient oliguria has also been reported to occur with constriction of the renal vein [11]; it has been shown that similar hemodynamic changes are induced in oliguric renal failure [12]. It is logical to suppose that complexes will bypass the glomeruli when this happens, with diminished amounts being deposited in these structures.

Regardless of the mechanism responsible for these differential changes, the results of the study bear upon clinical events. It informs us that the superimposition of renal vein thrombosis on a patient with preexistent or concurrent immune-complex-mediated nephritis will not aggravate this process and, in fact, may have a temporarily beneficial effect. From a practical standpoint, it would suggest that a renal biopsy may be better performed on the kidney not affected by this form of vascular pathology, because the severity of histologic change may be masked. Finally, it must be speculated as to whether an increase in venous pressure from other causes such as congestive cardiac failure may also diminish the tendency for immune complexes to be sequestered in the kidney. More studies are required to define the various physiologic influences that influence the localization of complexes in the glomerulus.

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